

WHAT IS CLAIMED IS

- 5 1. A method for detecting a nucleic acid, the method comprising:
contacting a first nucleic acid to a second nucleic acid, which second
nucleic acid comprises a neutral or positively charged fluorescent label; and,
detecting fluorescence polarization of the resulting mixture of first and
second nucleic acids.
- 10 2. ~~The method of claim 1, wherein the fluorescence polarization is
increased by less than about 50% by the addition of polylysine to the first and second
nucleic acid.~~
3. The method of claim 1, wherein the mixture of first and second
nucleic acids is present in a composition which is substantially free of polyion.
4. The method of claim 3, wherein the composition comprises less
than 1 μ M polyion.
- 15 5. The method of claim 1, wherein a rotational diffusion rate of a
duplex of the first and second nucleic acid is less than a rotational diffusion rate of the
first or second nucleic acid.
6. The method of claim 5, wherein the fluorescence polarization of
unduplexed first or second nucleic acid is at least 50% different than the fluorescence
polarization of the duplexed nucleic acid.
- 20 7. The method of claim 1, wherein the first or second nucleic acid
comprises one or more of: DNA, RNA, LNA, a DNA analogue, an RNA analogue or
a PNA.
8. The method of claim 1, wherein one or more of the nucleic acids
is nuclease resistant.
- 25 9. The method of claim 1, wherein the fluorescent label comprises
rhodamine or BODIPY.

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10. The method of claim 1, wherein the first nucleic acid is a DNA and the second nucleic acid is a PNA which comprises a rhodamine label.

11. The method of claim 1, wherein the first or second nucleic acids comprise at least a region which is single-stranded.

5 **12.** The method of claim 11, wherein the first and second nucleic acid are perfectly complementary.

13. The method of claim 11, wherein the first and second nucleic acid comprise at least one non-complementary nucleotide when aligned for maximum complementarity.

10 **14.** The method of claim 11, further comprising determining from the fluorescence polarization detection whether the first and second nucleic acids are duplexed.

15. The method of claim 11, further comprising determining the extent to which the first and second nucleic acids are duplexed from the fluorescence polarization detection.

16. The method of claim 1, wherein the first and second nucleic acids hybridize in solution prior to detection of fluorescence polarization.

17. The method of claim 16, comprising comparing the detected fluorescence polarization to a fluorescence polarization measurement of either the first or the second nucleic acid alone in solution.

18. The method of claim 16, comprising comparing the detected fluorescence polarization to a fluorescence polarization measurement of either the first or the second nucleic acid hybridized to a third nucleic acid.

19. The method of claim 18, wherein the third nucleic acid is perfectly complementary to either the first or the second nucleic acid.

20. The method of claim 18, wherein the third nucleic acid is not perfectly complementary to either the first or the second nucleic acid.

21. The method of claim 18, wherein the third nucleic acid is unrelated in sequence to either the first or the second nucleic acid.

22. The method of claim 16, comprising detecting fluorescence polarization during hybridization of the first and second nucleic acid.

5 23. The method of claim 22, further comprising determining the fluorescence polarization as a function of time during hybridization of the first and second nucleic acid.

24. The method of claim 23, further comprising plotting a histogram of the fluorescence polarization as a function of time.

10 25. A method of identifying the presence of a subsequence of nucleotides in a target nucleic acid, the method comprising:

contacting the target nucleic acid sequence with a labeled nucleic acid probe, which labeled nucleic acid probe comprises a neutral or positively charged label comprising a fluorophore to form a first reaction mixture; and,

15 detecting the level of fluorescence polarization of the first reaction mixture.

26. The method of claim 25, wherein the target nucleic acid sequence comprises at least one locus for a single nucleotide polymorphism.

20 27. The method of claim 26, wherein the nucleic acid probe is complementary to one allele of the single nucleotide polymorphism in the target nucleic acid sequence.

25 28. The method of claim 25, comprising contacting a plurality of additional target nucleic acids with a plurality of additional labeled nucleic acid probes, which additional labeled nucleic acid probes individually comprise a neutral or positively charged label comprising a fluorophore to form a plurality of additional reaction mixtures; and,

detecting the level of fluorescence polarization of the plurality of additional reaction mixtures.

29. The method of claim 28, wherein the plurality of additional target nucleic acids individually comprise at least one locus for a single nucleotide polymorphism.

30. The method of claim 29, wherein the plurality of additional nucleic acid probes are individually complementary to at least one allele of each of the single nucleotide polymorphisms in the plurality of target nucleic acid sequences.

31. The method of claim 30, wherein the plurality of additional target nucleic acids are derived from a single species, variety, cultivar, cell, virus, or organism.

32. The method of claim 31, wherein identification of the single nucleotide polymorphisms provides a single nucleotide polymorphism genotype for the species, variety, cultivar, cell, virus or organism.

33. The method of claim 25, wherein the fluorescence polarization is increased by less than about 50% by the addition of polylysine to the target and probe nucleic acids.

34. The method of claim 25, wherein the target and probe nucleic acids are present in a composition which is substantially free of polyion.

35. The method of claim 34, wherein the composition comprises less than 1 μ M polyion.

36. The method of claim 25, wherein a rotational diffusion rate of a duplex of the target and probe nucleic acids is less than a rotational diffusion rate of the target or probe nucleic acids.

37. The method of claim 36, wherein the fluorescence polarization of the probe which is duplexed to the target is at least 50% different than the fluorescence polarization of the probe when not duplexed to the target.

38. The method of claim 25, wherein the target or probe nucleic acids comprise one or more of: DNA, RNA, LNA, a DNA analogue, an RNA analogue or a PNA.

39. A system comprising:

a container comprising a duplexed nucleic acid disposed in the container, wherein at least one strand of the nucleic acid duplex comprises a neutral or positively charged fluorescent label;

5 a polarized light source positioned to shine plane polarized light through a portion of the container, thereby exciting the fluorescent label during operation of the system; and,

a detector that detects resultant polarization of light emitted by the fluorescent label.

10 **40.** The system of claim 31; wherein the container comprises a microfluidic device which contains the duplexed nucleic acid in one or more channels or chambers of the device.

15 **41.** The system of claim 40, the microfluidic device comprising a body structure, the body structure having two or more intersecting microchannels disposed therein, the microfluidic device further comprising a source of the first nucleic acid and a source of a second nucleic acid, which sources are in fluid communication with the at least two intersecting microchannels, wherein, during operation of the device, the first nucleic acid is flowed from the source of the first nucleic acid into at least one of the at least two intersecting channels and the second
20 nucleic acid is flowed from the source of the second nucleic acid into the at least one channel, whereby the first and second nucleic acids are mixed in the at least one channel.

42. The system of claim 41, wherein the detector is proximal to the at least one channel.

25 **43.** The system of claim 31, wherein the fluorescence polarization is increased by less than about 50% by the addition of polylysine to the duplexed nucleic acid in the container.

44. The system of claim 31, wherein the duplexed nucleic acid is present in a composition which is substantially free of polyion.

45. The system of claim 44, wherein the composition comprises less than 1 μ M polyion.

46. The system of claim 31, wherein a rotational diffusion rate of the duplexed nucleic acid is less than the rotational diffusion rate of a first or second strand of the duplexed nucleic acid.

47. The system of claim 31, wherein the first or second nucleic acid comprises one or more of: DNA, RNA, a DNA analogue, an RNA analogue or a PNA.

48. The system of claim 31, wherein one or more of the nucleic acids is nuclease resistant.

49. The system of claim 31, wherein the fluorescent label comprises rhodamine or BODIPY.

50. The system of claim 31, wherein the first nucleic acid is a DNA and the second nucleic acid is a PNA which comprises a rhodamine label.

51. A microfluidic fluorescent polarization nucleic acid analysis system comprising:

a microfluidic device comprising a body structure having at least two microfluidic channels disposed therein;

a source of a first nucleic acid;

a source of a second labeled nucleic acid, which second nucleic acid comprises a neutral or positively charged fluorescent label;

a source of plane polarized light, which source is positioned to illuminate a portion of at least one of the at least two microchannels; and,

a fluorescence polarization detector positioned to detect plane polarized light emitted from the microfluidic device.

52. In an assay system for quantifying a nucleic acid hybridization parameter, which assay system comprises providing a first nucleic acid composition, which nucleic acid composition comprises a first nucleic acid having a positive or neutral fluorescent label, introducing a second nucleic acid into the first nucleic acid

composition to produce a second nucleic acid composition, the second nucleic acid reacting with the first nucleic acid to produce a fluorescently labeled product having a substantially different rotation rate than the first nucleic acid, a computer implemented process, comprising the steps of:

5 determining a first level of fluorescence polarization of the first nucleic acid composition;

 determining a second level of fluorescence polarization of the second nucleic acid composition;

10 comparing the first and second levels of fluorescent polarization; and,
 calculating the nucleic acid hybridization parameter.